Experimental Study of Sutureless Colorectal Anastomosis

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ABSTRACT

Background/Aims: The present research project has been made mainly with the idea of comparing the tensile strength values and histological answers of three types of colon anastomosis: sutured with silk 5/0; polyglycolic acid 5/0; and sutureless anastomosis with human fibrin gum.

Methodology: One hundred and five (105) Wistar breath rats allocated into 3 groups of 35 animals were used to implement this experimental research project: silk, polyglycolic acid and human fibrin gum. Furthermore, each group was subdivided in 5 series respectively to carry out an experimental study on the tensile strength parameter and anatomic-pathological determinations on the 10th, 20th, 30th, 40th and 50th day after the surgical intervention. The following surgical interventions were practiced on them: A cross section of the colon, followed by: group 1: an end-to-end discontinuous suture anastomosis with Silk; group 2: an end-to-end discontinuous suture anastomosis with polyglycolic acid; group 3: sutureless anastomosis with human fibrin gum. On the 10th, 20th, 30th, 40th and 50th days we proceeded to measure the anastomosis’ tensile strength value for each series. We used a tensile strength apparatus and waited until the break down of the suture sample took place and wrote down the value, in g/cm, given by the voltmeter at that moment.

Results: The results obtained indicate that anastomosis made in group 1 (silk) lasted longer than the tensile strength apparatus; followed by those practiced in group 2 (polyglycolic acid); and finally anastomosis carried out in group 3 (human fibrin gum). However in the anastomotic process carried out with the human fibrin gum the healing started from the 10th day.

In the same period of time we carried out the following anatomic-pathological determinations: a) sharp inflammation; b) edema; c) non-specific chronic inflammatory infiltrate; d) granulomatous inflammatory infiltrate to foreign bodies; e) fibrosis.

Conclusions: The results show a better answer for anastomosis made with human fibrin gum than those carried out with the two other suture materials. This conclusion is based on the facts that the human fibrin gum used to carry out sutureless anastomosis during this research project generated a lower sharp inflammation and speediness in its absorption; absence of granular reaction to a foreign body; a minor or non-existent edema at all; as well as a good fibrous healing speediness process.

Therefore, all these experimental results lead us to conclude that the human fibrin gum used to carry out sutureless anastomosis may be an alternative to the handmade conventional anastomosis. Moreover they are easy to be implemented.

INTRODUCTION

Anastomotic dehiscence and leakage are still the most important cause of morbidity and mortality during the period of healing of colorectal anastomosis. Nowadays, anastomotic dehiscence complications following a surgical procedure are located between 0.1 and 30%. When the two cut ends of the intestine (bowel) are sutured together the healing begins with an inflammatory reaction. Reaction that provokes the replacement of damaged cells by knitting of granulation and fibroblasts and later on by the deposit of conjunctive knitting that requires reconstructing the intestinal wall. The healing of the colon starts with a varying level of collagen in the submucous layer, where its concentration is the highest. The early prevalence of collagenolysis with the increase in collagen activity, followed by subsequent prevalence of collagen synthesis, are two opposing forces that will determine the period of healing of intestinal anastomosis. The colon continues the same repair process as the rest of the intestine, however, with minor loss of collagen and much faster reestablishment of itself. The most critical period of the healing is the first 3-5 days. During this time there is an invasion of the colon anastomosis by plackets, macrophages, granules and fibroblasts. This invasion leads to an increase in the collagenolysis and a decrease in the collagen activity, which infers that the colic anastomosis will be mainly held together by the uniting suture (1).

Human fibrin gum has proved to be a good adhesive that protects the intestinal anastomosis.

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Tested by several authors (2-4). Human fibrin gum has become a great utility in animal experimentation in which sutureless anastomosis was carried out, with the objective of eliminating the aggression that foreign elements to the human body provoke on the evolution of the anastomosis process.

We have studied the maximum supporting tensile strength value of sutureless anastomosis made with human fibrin gum in comparison with other conventional sutures (silk and polyglycolic acid). Furthermore, we have explored the requirements of tissue synthesis by different types of anastomosis throughout the determination of diverse anatomic-pathological parameters.

The main objective of this research project is to check out the human fibrin gum viability in the anastomotic process. Through the study of two parameters, tensile strength and the anastomotic wound healing in lab animals, we will try to validate the hypothesis that human fibrin gum is useful in the sutureless anastomotic process without suture.

METHODOLOGY

Materials

Lab animals: A) Anastomotic materials; B) Human Fibrin Gum heater; C) Tensile Strength Tester.

A) Animals

The current experimental study has been carried out with a total of 105 white rats, female, around 200g in weight and Wistar breath. Twenty-four hours previous to the surgical intervention animals were kept fasting allowing them to get water "ad libitum." After 24 hours from the surgical intervention, the intervened animals were allowed to be fed with their normal diet.

Experimental animals (Wistar rats) were allocated randomly into 3 groups of 35 animals. To proceed forward each group was divided in 5 subgroups of 7 animals, respectively, depending on the time between the 1st and the 2nd surgical intervention. In such a way groups were formed as follows: Group 1: silk 5/0: 35 animals. Series 1: 10 days; Series 2: 20 days; Series 3: 30 days; Series 4: 40 days; Series 5: 50 days.

Group 2: Polyglycolic acid 5/0: 35 animals with same series as above.

Group 3: Human fibrin gum: 35 animals and with equal series as the two previous groups.

B) Anastomotic Materials

Three types of anastomotic suture materials were used: Silk 5/0, polyglycolic acid 5/0 and human fibrin gum.

C) Human Fibrin Gum Heater (FIBRINO-THERM)

The Fibrinotherm is a device that combines a heater body with a system of magnetic agitation that favors the preparation of human fibrin gum.

Tensile Strength Tester: We have used a group of four gauges placed in the form of Whetstone’s bridge, that is housed on a flexible metal fledge. Gauges are placed in such a way that permits the compensation of the section that makes reference to the Poisson’s principle. The reading in volts, on the meter, is converted by means of a conversion factor in kilogram-power that corresponds to the tensile strength traction applied in the rehearsal (Figure 1).

Methods

Methods were divided into: 1) Anesthetic methods; 2) Surgical methods; 3) Getting samples and measuring the suture tensile strength methods; 4) Anatomic-pathological methods; 5) Statistical methods and; 6) Studies of mortality.

1) Anesthetic methods: All animals were anesthetized with the same technique previous to the surgical intervention. Each one was injected with a dose of 0.3cm³ of interperitoneal Ketamina per 100g of weight. Same technique was used to obtain samples.

2) Surgical methods: Surgical interventions carried out for groups with silk 5/0 and polyglycolic acid 5/0 were the same. By contrast surgical intervention for group with human fibrin gum differed in the anastomotic technique applied to the sectioned colon.

2a) Silk 5/0 (S) group: After making a half laparoscopy, the external intestinal handles came out. Once the descending colon was located, we proceeded to practice a cross section of it followed by an end-to-end anastomosis with silk 5/0 and a discontinuous suture. The closing of the abdominal wall (chest cavity) was carried out with silk 2/0 using a continuous suture in block of the peritoneal cavity, aponeurosis and skin separately.

2b) Polyglycolic acid group (APG): This group suffered similar interventions to those that were carried cut on the previous group.

2c) Sutureless Human Fibrin Gum Group (SFHG); In this group, the technique was similar to the two previous groups. However after practicing a cross section of the colon, the two cut ends of the bowel were sutured with three points of polyglycolic acid, in order to obtain a triangular and closer position of the end sections. To carry out a complete sealing of the union the human fibrin gum was placed on the three lines that came out from the union of the cut ends (Figure 2).

![Figure 1: Tensile strength apparatus.](image-url)
markings. (This segment was placed and fixed onto the tensile strength apparatus by two jagged gaps that impeded any slide of the sample.) Finally with the tensile strength apparatus turned on we waited for the break down of the suture sample and wrote down the value, in g/cm, given by the voltmeter at that moment.

4) Anatomic-pathological methods: Once the break down of the suture took place, and its tensile strength value was measured, the left portion of the fragments used to fix the ends of the intestinal anastomotic segment were extirpated. The rest of the intestinal anastomosis was placed into formal 10% buffered for its anatomic-pathological study. The 3-µ thickness histology cuts were processed in paraffin and finally stained with the usual hematoxylin and eosin (H&E) technique. They were also stained using Masson’s trichrome stain so that the connective tissue could be evaluated.

The studied elements were: presence of inflammatory cells; types of inflammation: sharp, chronic: non-specific; granulomatous reaction to foreign bodies; edemas; fibrosis (Figures 3 and 4).

5) Statistical methods: Based on the previously obtained results we proceeded to make several statistical studies. We accomplished an analysis of the variance with all the results from each parameter, with the purpose of checking if any difference between the groups existed. We also studied the correlation between pairs throughout the test of Mann-Whitney. This is a non-parametric for comparing the homogeneity of two media in the case of different or independent samples with significance values of 5% (P<0.05).

6) Mortality study: Carried out to determine the surviving animals after the intervention. All animals from each group were observed according to the days lapsed from the intervention or from the taking of tensile strength values, appreciating the occurring deaths.

RESULTS
A) Results from the Tensile Strength Tester (Figure 5).

The results obtained indicate that anastomosis made in Group 1 (Silk) lasted longer than the tensile strength apparatus; followed by those practiced in Group 2 (Polyglycolic Acid); and finally anastomosis carried out in Group 3 (Human fibrin gum). However in the anastomotic process carried out with the human fibrin gum the healing started from the 10th day.

1. Colic anastomosis with silk 5/0.
   Values from the five series, group I: series I: 248.9; series II: 345.6; series III: 304; series IV: 407.7; series V: 436.5.

2. Colic anastomosis with polyglycolic acid 5/0.
   Values from the five series, group II: series I: 275; series II: 300; series III: 285.8; series IV: 311.6; series V: 321.3.

3. Colic anastomosis with human fibrin gum.
   Values from the five series, group III: series I: 180; series II: 255; series III: 261.6; series IV: 255; series V: 316.
B. Anatomic-pathological results

The studied elements were: a) Sharp inflammation; b) Edemas, c) Inflammation Chronic; d) Granulomatos inflammatory infiltrate to foreign bodies; e) Fibrosis. The results show a better outcome for anastomosis made with human fibrin gum than those carried out with the two other suture materials. This conclusion is based on the facts that the human fibrin gum used to carry out sutureless anastomosis during this research project generated a lower sharp inflammation and speediness in its absorption; absence

| TABLE 1 Anatomico-pathological Results - Colonie Anatomoses with Silk |
|--------------------------|------------------|------------------|------------------|------------------|------------------|
| S.I. | E | NCII | GIF | F |
|--------------------------|------------------|------------------|------------------|------------------|------------------|
| Series I (D-1). Anatomico-pathological results at 10 days | 1.43 | 0.57 | 0.88 | 1.43 | 1.14 |
| x | 0.17 | 0.00 | 1.33 | 1.50 | 1.67 |
| Series II (D-11). Anatomico-pathological results at 20 days | 0.14 | 0.00 | 0.56 | 0.25 | 0.22 |
| x | 0.50 | 0.00 | 0.83 | 1.50 | 1.67 |
| Series III (D-111). Anatomico-pathological results at 30 days | 0.25 | 0.00 | 0.47 | 0.25 | 0.22 |
| x | 0.17 | 0.00 | 1.50 | 1.17 | 2.00 |
| Series IV (D-IV). Anatomico-pathological results at 40 days | 0.14 | 0.00 | 0.25 | 0.58 | 0.00 |
| x | 0.33 | 0.00 | 1.33 | 1.83 | 2.17 |
| Series V (D-V). Anatomico-pathological results at 50 days | 0.22 | 0.00 | 0.22 | 0.14 | 0.47 |
| x | 0.00 | 0.00 | 1.67 | 0.83 | 2.60 |
| Series VI (D-VI). Anatomico-pathological results at 60 days | 0.09 | 0.00 | 0.22 | 0.47 | 0.25 |
| x | 0.00 | 0.00 | 1.67 | 0.83 | 2.60 |
of granular reaction to a foreign body; a minor or non-existent edema at all; as well as a good, speedy fibrous healing process.

B1. Anatomic-pathological results - colonic anastomoses with silk (Table 1).

B2. Anatomic-pathological results - colonic anastomoses with poliglicolic A. 5/0 (Table 2).

B3. Anatomic-pathological results - colonic anastomoses with human fibrin sealant (Table 3).

DISCUSSION

Medical surgery has been developed to acquire diverse, more reliable and safer intestinal anastomotic techniques, as well as minor risk of complications after a surgical intervention. Diverse procedures exist that try to preserve anastomosis so that they avoid possible anastomotic dehiscence or leakage, getting a correct healing. When the two cut ends of the intestine are sutured together is the most critical period of the healing. The early prevalence of collagenolysis with the increase in collagen activity, followed by subsequent prevalence of collagen synthesis, are two opposing forces that will determine the period of healing of intestinal anastomosis, as it is mainly held together by the uniting sutures. At this time the surgical technique and the material employed acquire a great importance, since the strength and resistance of the suture depend on them.

The healing of an intestinal anastomosis, as well as its strength and resistance, obey to a great degree what histologically may happen on the suture line. There are also many other factors that may compromise the healing of the anastomosis, such as: the surgical technique, the vascular technique, the fecal content, the infections, bacterial endotoxines, radiotherapy, corticosteroids, etc. (5-8). It is known that the tensile strength of the colon recovers much faster than that of the skin, since the strength of the newly formed collagen catches up very soon with that of the old collagen (6). Biological adhesives have been used to seal anastomosis practiced with different suture materials (9) and, to an experimental level, to carry out sutureless anastomosis (10,11).

This project aims to check out the human fibrin gum viability in the colon anastomotic process, through the study of sutureless anastomosis made with human fibrin gum in comparison with other conventional sutures (silk and polyglycolic acid in experimental animals. The conclusions of this project are based on the results obtained from the study of the tensile strength that sutures are able to support and on the anatomic-pathological consequences related to the anastomotic wound healing such as sharp inflammation, edema, non-specific chronic and granulomatous inflammatory infiltrate reaction to foreign bodies and the anastomotic fibrosis.

Galletti et al (12) used fibrin adhesive in colon anastomosis in pigs, concluding that anastomosis carried out with this adhesive material did not need suture, favoring the knitting union within the first 30 days from the surgical intervention. On the other hand, these authors say that suture materials seem to be highly limited for the knitting union, and at the same time they favor a more durable inflammation because of cellular infiltration.

Given the methodology carried out in this research, we could affirm that from the first 10 days, conditions exist that favor the union of the anastomotic cut ends. Contrarily to the study carried out by Galletti et al we have observed macroscopically that the human fibrin gum tends to stick the knitting together and that macroscopically there is a great presence of inflammatory cells.

Jansson et al (10) compared the sutureless colon anastomotic healing on 10 pigs using a modified stapler without staples to make the application of the human fibrin contrarily to the sutures carried out with EEA stapler and with handmade monoplane ones. We considered our project as a better job to the Jansson et al (10) not only for the number of animals used and its pursuit during the project but also because these authors sacrificed the animals on the 4th day.

Detweiler et al (2) have published a new sutureless anastomotic technique using a non-toxic, absorbable endoluminal slippery stent (SAINT) with human fibrin gum compared with handmade monoplane anastomotic sutures. Made from heated sucrase, water and handily introduced in aluminum molds the SAINT dissolved itself between 30 and 60min after its application. This project involved 57 pigs, in which they mainly and simultaneously carried out a colon and thin intestinal anastomosis. It doesn’t seem to be a very high quality and developed method. Comparing Detweiler et al (2) project with the current one, we find the latest much more orthodox not only for the number of animals involved but also for the development of individual techniques that make it more reliable on it.
results.

The results obtained in our experimental project allow us to carry out a series of comments based on a deep and thorough analysis of the collected data on the parameters we have studied.

The sutureless anastomosis tensile strength values in the human fibrin gum group increased progressively from the first 10-50 days, not existing significant differences between the values obtained from the 30th-50th day.

We agree with Galletti et al (12) in the fact that anastomosis carried out with human fibrin gum didn’t need suture in the studied animals. However we differed in the time needed for the anastomotic healing. They affirm that the healing of the anastomosis takes place within the first 30 days from the surgical intervention. In our study we have reached the conclusion that within the first 10 days after the two cut ends of the intestine are sutured together the anastomosis starts healing. Notwithstanding, it is true that the tensile strength of the anastomosis increases later on after the first 10 days.

Comparing the tensile strength results obtained in the human fibrin gum group with those that came out from the silk group, we found that initially the first group showed lower values than the second one. However, in relation to the polyglycolic acid group results the tensile strength values in the human fibrin gum group went progressively up in such a way that within the first 40-50 days tensile strength differences between both groups’ figures did not exist. We also need to mention that the silk group values were higher than the polyglycolic acid group values.

Therefore, between our study and that by Galletti et al (12) statements differ about the fact that suture materials properties seem to be limited in the healing process, as the silk reaches a greater tensile strength than the human fibrin gum. Moreover, we disagree with Kjaergaard et al (13) who affirm that they don’t find significant differences in the anastomosis strength with the passing of the time. By contrast, we agree with Jansson et al (7) in the fact that handily sutured anastomosis show higher tensile strength than those carried out exclusively with human fibrin gum.

Statistically it is relevant that after the first 10 days the human fibrin gum caused a minor sharp inflammatory reaction compared to the silk. On the other hand within the first 20 days there were not significant differences between both groups. However within the 30, 40 and 50 days again apparent statistical disparities came out with minor inflammatory reaction on anastomosis carried out with the human fibrin gum.

Comparing the tensile strength histology parameter results obtained for the human fibrin gum group and the polyglycolic acid any significant differences appeared, except from the IV series (40 days) values where the latest (polyglycolic acid) presented a more marked reaction than the first one (human fibrin gum). Whereas the purpose of this research project is not to compare the results obtained for the silk and polyglycolic groups, it is important to mention that statistically the sharp inflammation was always more intense in the silk 5/0 series than in polyglycolic acid series.

These results agree with the conclusions of Galletti et al (9) that suture materials seem to favor a more durable inflammation caused by the higher cellular infiltration.

Certain contradictions between Jansson et al’s (7) discoveries and the current research may exist, since they find lower levels of blood flow in handmade anastomosis with suture materials than the levels obtained in sutureless anastomosis carried out with human fibrin gum.

While no edema appeared in the human fibrin gum and polyglycolic acid groups, they came out in the silk 5/0 group. Nevertheless the edema in the silk 5/0 group went progressively down to disappear within the 50 days, having no significant differences between the results obtained in different periods of time.

In relation to the non-specific chronic inflammatory infiltrate parameter (IICI) in the human fibrin gum group after an increase up to the 20th day and a later decrease until the 500th day, non-significant differences between both values appeared. The IICI took place in each one of the groups of silk 5/0 series, reaching its higher value by the 40th day, with no significant differences between them. In APG group the IICI also appeared in each one of the observed periods going up to its top value on the 30th and 40th day, without apparent statistical inequalities.

After the first 10 days the SFHG group showed IICI values with non-significant differences compared with those IICI obtained in the S group. However, this histologic parameter (IICI) resulted slightly higher in the first (SFHG group) than in the second (S group). Within the 20, 30, 40 and 50 days the animals intervened using human fibrin gum suture showed an IICI values similar to the results obtained in the silk group in identical periods.

Although the IICI went down accordingly to the day’s lape it was more evident in the CFH group than in the S group. However there were not significant statistic differences between both groups.

In the APG group significant differences did not appear for the IICI parameter with regard to the SFHG group, apart from the 20th day where the results with APG were significantly lower than those with SFHG. By contrast, with non-statistical relevant meaning, the APG group IICI values were higher than the SFHG on the 50th day.

Regarding the chronic granulomatus inflammatory infiltrate to foreign bodies parameter (IICG) in the SFHG group there was no answer to this histology parameter in any of the groups. It is easy to understand since the SFHG is a material that doesn’t provoke any answer to a foreign body even when we have worked with human fibrin gum in lab animals like the rat. However, we found an IICG positive answer in the S group in each one of the observed time periods, initially with lack of meaning between them, but being more evident later on.

In the APG group all the series presented an IICG, reaching this histology parameter its higher value on
the 40th day, although without significant differences among the series.

Comparing IICG results between the SFHG and the S groups, they were always statistically significant. In general, it happened to be equal to the correlation between the APG and the SFHG groups, notwithstanding, reached the 30th and the 50th day with no differences appearing.

Relating to the fibrosis parameter we observed how the healing of the anastomosis in the SFHG group was progressively getting compactness until the 40th and 50th day, not finding, however, significant differences between the series. This fact agrees with Galletti et al’s statement, that the line of human fibrin gum induces the union of the knitting and becomes a reliable repairer of the same.

In the S group the linking knitting was increasing with postoperative time, without significant differences between the series. In the APG group, this parameter increased in time after the surgical intervention reaching its highest value at the 50th day.

The fibrosis parameter was mainly more apparent in most of the SFHG group than in the S group, being the 50th day when the linking knitting was more compact (dense collagen), although significant differences didn’t exist between them. Between the results of the SFHG group and those obtained for the APG group no inequalities appeared.

Jansson et al (10) didn’t find significant collagen concentration differences on the 4th day between the animals with anastomosis carried out with staples and those made with human fibrin gum or even with handmade suture; however, this concentration was something lower in the first of the groups. It seems to agree with our results, given the almost null statistical differences found in the fibrosis parameter on the 10th day between the 3 groups.

During the observation period, we have come out with the fact that the mortality in any of the three groups, although lower for the S group, didn’t show significant differences with the others (S: 11.42%; APG: 14.28%; SFHG: 14.28%). Six deaths caused by anesthetic problems were observed right after the surgical intervention. Whereas a wall dehiscence and a sepsis for abscesses of the wall took place in the S group, they didn’t have a significant value in the group as a whole. The APG group presented an anastomotic dehiscence and a subsequent fecal peritonitis and another animal within this group died without a clear cause. It was quite remarkable the presence of three clogging clinical diagnostics (8.57%) in the SFHG group. We can infer, upon the appreciated adherence as the cause of the clogging that the SFHG tends to generate clogging risks in higher proportion than other suture materials.

All these experimental results lead us to conclude that the human fibrin gum used to carry out sutureless anastomosis may be an alternative to the handmade conventional anastomosis. Moreover they are easy to carry out; generate a lower sharp inflammation and chronic granulomatous reaction; a minor edema as well as a good fibrous healing. Though, the tensile strength values of this type of anastomosis are not higher when compared with the two other studied materials (silk 5/0 and polyglycolic acid 5/0).

We are, therefore, either in line with the results obtained by Galletti et al (12) who conclude that to an experimental level anastomosis carried out with human fibrin gum do not need suture; or with Jansson et al (10) who affirm that the results of their experimental study indicate that sutureless colic anastomosis, in animals, can be carried out with a simple technique and obtaining some reliable results.

In the very same sense and based on their SAINT method Detweiler et al (2) published in 1995 that the human fibrin gum could be an effective anastomotic method to treat the colon without suture. More recently, Trignano et al (4) have also found a similar process of healing in rats only treated with human fibrin gum in front of those treated with absorbable suture material.

REFERENCES


